VICH Biological Quality Monitoring WG
Topic: Residual Formaldehyde Testing

Collaborative Study Protocol

Performance Characteristic Determination
Ferric Chloride Method for Residual Formaldehyde
Proposed VICH Guideline

Purpose:
To determine the performance characteristic of the VICH proposed test for residual formaldehyde (Ferric Chloride Method, Appendix 1) as conducted by industry and government reference labs in Japan, Europe, the United States and observer countries.

Testing plan overview:
Determining the performance characteristics of the proposed ferric chloride residual formaldehyde test will take place in two phases. The first stage study will consist of the distribution of samples based on three current commercial products to the three regions. These three products include: 1) a *Clamydi*a (inactivated) vaccine, 2) a *Clostridium* bacterin-toxoid vaccine, and, 3) a *Pasteurella multocida*, avian origin bacterin vaccine

A maximum of 5 industry and government labs from each region (EU/US/Japan and official observer) will be selected by the WG representatives to participate in this study. Each participating lab will test the samples (blinded) by their current assay and the Ferric Chloride Method. Results will be collected by the regional government lab and submitted to the topic leader. These data will show the consistency of testing between regions over a range of residual formaldehyde levels and products.

Phase two would involve the evaluation of a minimum of 5 regional products, in duplicate using both the current regional assay and the Ferric Chloride Method. These data will demonstrate the utility of the proposed guideline assay for a variety of products, and provide preliminary data the consistency of the assay over a variety of products.

Materials and Methods:
Vaccines, phase 1: USDA- CVB-L has identified, obtained and re-vialed the three vaccines listed above. Coded samples of the three vaccines will be made available to the member (and observer) countries government test lab for further distribution.

The USDA will be responsible for sample distribution to the Japan Government Lab and to the EP. Government labs will be responsible for obtaining any import permits. USDA will be responsible for sample distribution to the government labs. The Japan Government Lab and the EP will be responsible for distribution to industry participants.

Current standard procedure:
Collaborators will also conduct tests according to their current standard method. They will provide details of the test procedure along with the results. Labs currently running the Ferric Chloride Method can provide that data only. Any variances in test procedure from the proposed VICH guideline should be identified.

Collaborative Instructions:
Detailed instructions will be distributed with the collaborative samples, including: names and addresses of coordinators, instructions on methods, checklists, reporting forms, and other information.

Results:
All results should be reported to the region (or observer) coordinating lab. From there, results will be forwarded to the topic leader for analysis and reporting.. The following conversion table is provided for clarification:

g/L formaldehyde	% w/v formaldehyde	% v/v formaldehyde solution*	ppm formaldehyde
2.0	0.2	.5	2000
0.8	0.08	0.2	800
0.4	0.04	0.1	400
0.5	0.05	0.125	500
0.05	.005	0.0125	50
0.04	.004	0.01	40

*based on 40% formaldehyde solution

Timeline:

March 21, 2000	WG forwards draft collaborative protocol to WG members
April 1, 2000	USDA distributes collaborative samples and materials through coordinators
May 26, 2000	Collaborators complete all testing and report
June 2, 2000	Data analysis completed
June 9, 2000	Report of study results to members
July 11-14, 2000	WG meeting

Appendix 1: Ferric Chloride Method

1. **Reagents**
   - 1.1. *Ferric chloride-sulphamic acid reagent.* A solution containing 10 g/l of *ferric chloride* and 16 g/l of *sulphamic acid*
   - 1.2. *Methylbenzothiazolone hydrazone hydrochloride.* (MW 233.7). [CAS 149022-15-1]. 3-Methylbenzothiazol-2(3H)one hydrazone hydrochloride monohydrate. An almost white or yellowish, crystalline powder. mp: about 270 °C.
     - *1.2.1. Suitability for determination of aldehydes.* To 2 ml of *aldehyde-free methanol add* 60 µl of a 1 g/l solution of *propionaldehyde in aldehyde-free methanol* and 5 ml of a 4 g/l solution of methylbenzothiazolone hydrazone hydrochloride. Mix, allow to stand for 30 min. Prepare a blank omitting the propionaldehyde solution. Add 25.0 ml of a 2 g/l solution of *ferric chloride* to the test solution and to the blank, dilute to 100.0 ml with *acetone R* and mix. Measure absorbance of the test solution on a spectrophotometer at 660 nm in a 1 cm cell using the blank as compensation liquid. The absorbance of the test solution must be greater than or equal to 0.62 absorbance units.
   - 1.3. *Formaldehyde solution*, Contains not less than 34.5 per cent w/v and not more than 38.0 per cent w/v of formaldehyde (CH_2O)
   - 1.4. *polysorbate 80,* analytical grade
   - 1.5. *isopropyl myristate* analytical grade
   - 1.6. *hydrochloric acid*, (1 M) analytical grade
   - 1.7. *chloroform,* analytical grade
   - 1.8. *sodium chloride* (9 g/L solution), analytical grade
   - 1.9. *polysorbate 20,* analytical grade

2. **Sample and Standards Preparation**
   - 2.1. Prepare *formaldehyde* standards of 0.25, 0.50, 1.00and 2.00g/L by diluting *formaldehyde solution* (1.3) in suitable volumetric flasks.
   - 2.2. If the vaccine to be examined is an emulsion, separate the aqueous phase by the following method. Add to the vaccine an equal volume of *isopropyl myristate and* mix. To 3 volumes of the mixture add 2 volumes of 1 *M hydrochloric acid*, 3 volumes of *chloroform and* 4 volumes of a 9 g/l solution of *sodium chloride.* Mix thoroughly. Centrifuge at 15,000 g for 60 min. Remove the aqueous phase and measure its volume. Use the aqueous phase for the test for formaldehyde described above, adjusting the concentration of formaldehyde in the standard to allow for the dilution of the vaccine during the separation of the phases. If the procedure described fails to separate the aqueous phase, add 100 g/l of *polysorbate 20* to the sodium chloride solution and repeat the procedure but centrifuging at 22,500 *g*.

3. **Test method**
   - 3.1. To 0.5 ml of a 1 in 100 dilution of the vaccine and to be examined, and to the *formaldehyde* standards, add 5 ml of a 0.5 g/l solution of *methylbenzothiazolone hydrazone hydrochioride* and 0.05 ml *of polysorbate 80,* close the tubes, shake and allow to stand for 60 min.
   - 3.2. Add 1 ml of *ferric chloride-sulphamic acid reagent* and allow to stand for 15 min.
   - 3.3. Measure absorbance of vaccines and standards on a spectrophotometer at 660 nm in a 1 cm cell using the reagent blank as compensation liquid.

4.0 Calculations

Calculate total formaldehyde concentration (g/L) from the standard curve using simple linear regression. For a satisfactory test, total formaldehyde will not exceed 2.0 g/L formaldehyde . Higher levels may be justified case-by-case, based on a scientific need and supporting data.